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Saved by the Formulation: Tackling Aggregates and Viscous Challenges in High-Concentration Monoclonal Antibody Development

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Executive summary

This report explores the development of a high-concentration lyophilized monoclonal antibody formulation, aiming for treatment of pancreatic cancer. The formulation focuses on addressing critical challenges in stability, viscosity, and aggregation to facilitate subcutaneous administration.

Pancreatic cancer remains one of the deadliest malignancies, with a 5 year survival rate below 10%. Despite increasing monoclonal antibody approvals for various cancers, none specifically target pancreatic cancer. This project aims to develop a stable, multidose anti-HER2 mAb for subcutaneous delivery, ensuring enhanced patient compliance, accessibility, and healthcare efficiency.

The formulation contains excipients like sucrose, histidine, and proline in order to stabilize the mAb while minimizing viscosity and aggregation. Freeze-drying techniques optimize long-term stability, balancing solid content to prevent aggregation and enable effective reconstitution in saline.

The Structured Literature Review highlighted persistent industry challenges like protein cake stability, reconstitution time, and viscosity. Experiments identified an optimized formulation combining sucrose, proline, and polysorbate 20. Analytical evaluations, including pH titration, Dynamic Light Scattering, and concentration analysis, confirmed the formulation’s efficacy and stability.

The subcutaneous administration method is poised to disrupt the market by addressing unmet needs in patient convenience and healthcare system efficiency. The product adheres to GMP and ICH guidelines. The quality management system emphasizes robust traceability and product safety.

The manufacturing process integrates continuous production techniques, leveraging bioreactor systems and automated filling lines for scalable, cost-effective production. This innovation positions the product as a frontrunner in addressing pancreatic cancer's urgent therapeutic needs. In conclusion, the high-concentration lyophilized mAb formulation bridges critical gaps in cancer therapy by offering a stable, patient-friendly, and scalable solution, paving the way for life changing healthcare advancements.

Abbreviations

API: Active Pharmaceutical Ingredient

CEX: Cation Exchange Chromatography

AEX: Anion Exchange Chromatography

mAb: Monoclonal antibodies

HC: High Concentration

MD: Multidose

IV: Intravenous

SC: Subcutaneous

NDA: New Drug Application

ANDA: Abbreviated New Drug Application

IND: Investigational New Drug

SOM: Small Organic Molecule

MABEL: Minimum Anticipated Biological Effect Level

NOAEL: No Observed Adverse Effect Level

PMN: Premarket Notification

PMA: Premarket Authorization

IDE: Clinical Trial Exemption

EMA: European Medicines Agency

FDA: Food and Drug Administration

GMP: Good Manufacturing Practices

PFS: Pre-filled Syringe

CSTR: Continuous Stirred-Tank Reactor

MNC: Multi-National Company

DLS: Dynamic Light Scattering

XRPD: X-Ray Powder Diffraction

Content

1 Key Highlights

- Marketing should focus on where we have a competitive edge against similar drugs, eemphasizing on convinianc drug and less invasive route of delivery.
- Obtaining patent rights and market approval in the US and selected EU countries should be proitized, excluding competition, while attracting investors and giving the highest return on investment.
- The intended final product, the auto injector, offers the best convenience and patient compliance avaible for this type of drug.
- sucrose, proline, and polysorbate 20, as the best choice due to its excellent characteristics.
- Continuous production includes amongst others automated aseptic filling and continuous freeze-drying to maintain sterility, prevent moisture reabsorption, and ensure product stability, facilitating the optimal production conditions and safety of the drug.

2 Aim

This report aims to investigate the making of a high-concentration (HC) lyophilized, multidose, anti-HER2 monoclonal antibody (mAb) (Trastuzumab) formulation. The final product must have certain quality attributes in relation to patient compliance and competitive edge.

1. To formulate a lyophilized, high-concentration, multidose, anti-HER2 monoclonal antibody (mAb) for treating pancreatic cancer.
2. The product should be stable when reconstituted in 2 mL saline water for injection in a concentration range of 10 — 50 mg/ml.
3. Product design should be aimed at subcutaneous administration.
4. The product should “ideally” be produced using continuous production.

Furthermore, we aimed to assess the business potential for different regions and developed a marketing and intellectual property (IP) strategy around the drug, taking into account pharmaceutical regulations and IP licensing fees for different regions.

3 Background based on the NABC

Needs

As of 2021, no monoclonal antibodies (mAb) indicated for pancreatic cancers were approved on the market, despite the prognosis being worse than for many other malignancies. The five-year survival rate was less than 10%, in the same year.[1] Currently (2024) most of the mAb-based therapies available on the market, especially those indicated for various types of cancer including pancreatic, are administered intravenously (IV), underscoring the need for a subcutaneously administered mAb formulation.

Since mAbs are low-potent drugs, relatively high concentrations (> 50 mg/mL ≤200 mg/mL) are needed. A lyophilized *high concentration (HC),* multidose (MD) mAb formulation with subcutaneous administration would be the optimal solution, meeting compliance and convenience requirements[2]. Requirements currently not met by IV-administered drugs as patients must go to the hospital for treatment. This involves more work for healthcare staff and places a significant burden on patients in terms of time, travel, and potential lost productivity.

Approach

In developing a lyophilized HC Trastuzumab formulation, we prioritized excipient selection to optimize solubility while minimizing viscosity and aggregation risk. These factors are critical for successful lyophilized HC mAb formulations[3].

The focus has been on a crucial, yet often underestimated, element: cryoprotection. Specifically, we investigated the use of sucrose.[4] While sugars like sucrose are generally minimized in formulations due to their potential to increase viscosity, we recognized their significant ability to stabilize proteins and prevent degradation during freeze-drying. Sucrose provides cryoprotection by replacing the hydrogen bonds typically formed with water, thereby reducing stress on the mAb during the freeze-drying process[5].

To keep viscosity low, special attention was paid to the total amount of solubilized content, as over 10% solids on the formulation impede the drying step in freeze drying. With a limited concentration of solids, the sublimation process will be as efficient as possible, resulting in a long-term stable product with minimal moisture.

Furthermore, proline and/or histidine were added as viscosity-reducing agents, due to their protein-protein interaction reduction capabilities, and polysorbate 20 as surfactant, preventing aggregation of mAb[4–6].

Benefits

SC delivery allows for self-administration, significantly improving convenience and potentially increasing patient compliance and satisfaction with treatment. This method also offers advantages for healthcare providers by reducing the need for hospital visits, freeing up resources, and saving time.

Developing HC lyophilized formulations is a challenge across the pharmaceutical industry. The demand for such formulations presents a significant market opportunity. Successfully creating an HC lyophilized formulation with the quality attributes required for modern therapies would provide a substantial competitive edge.

Competition

As stated above, the market is concentrated around the interest of this type of formulation in a market with unrealized potential, hence most of the available drugs being IV. By beating competitors to the market with an aggressive business model we will take the lion share.

Our business approach offers several advantages. Firstly, our formulation is unique and does not infringe upon existing formulations. We have secured intellectual property rights of the formulation recipe to ensure a strong market position by attracting investors and eradicating early competition. To gain market share as quickly as possible we will out-license the patent to specialized subcontractors, securing a standing fee. Regulation and market approval processes and costs deviate between regions, thus, prioritizing those with the largest return on investments will maximize our chances of maintaining the patent and expanding to new regions.

Finally, Our formulation is relatively simple, and optimized for continuous production and subcontractors and licensing partners are quality-assured in accordance with international standards.

4 Scientific background

Epidemiology

Today, pancreatic cancer marks one of the deadliest cancers worldwide. For the past years, incidence and mortality rates of pancreatic cancer have shown an increase, marking a huge segment of customers worldwide.[7] When discovering pancreatic cancer, most individuals were already at an advanced stage. Estimations say that only about 12 % of patients have a survival time of more than 5 years. Therefore, it is concluded that pancreatic cancer is the deadliest of all cancers and treatments have never been more crucial for saving the lives of the patients while optimizing the treatment-process for the healthcare providers[8].

The market for cancer therapies continues to grow due to the increased prevalence of patients diagnosed with cancer worldwide. As of 2021, 45 mAbs have been approved in the USA and/or the European Union (EU) for the treatment of patients with a wide range of cancers[9]. However, no antibody-based drugs have been approved for the treatment of patients with pancreatic cancer[10].

The main curative treatment today is surgery, however only 15–20 % of tumors are resectable at the time of patient diagnosis, and the prognosis is still poor[11]. Some argue that the best approach for curing pancreatic cancer is combining surgery with chemotherapy. However, challenges such as high recurrence rates (70-80 %) and post-surgical complications limit its long-term success. In relation to the required sources in health care systems, hospital bed capacity etc the negative outlook increases as well[12,13].

Freeze drying

Freeze-drying is a method used to preserve medicines and other perishable material e.g. food[14]. By a three-step process, water is removed from the material, preventing microorganisms from spoiling it, including the prevention of protein aggregation and degradation. Freezing, primary and secondary drying are the three main steps. In the

first step — freezing — cooling the formulation below the glass transition temperature of the maximally freeze-concentrated solution (Tg′) is crucial. This parameter allows the amorphous formulation to maintain its structural integrity during the subsequent drying steps (sublimation). Conversely, crystalline formulations have other properties regarding drying or reconstitution, not always favorable. Nucleation is another important physio-chemical factor during freezing that has implications in the drying steps. It affects the sublimation rate and final moisture content. An annealing step (raising the temperature slightly above T_g at the end of freezing stage) can help make a more homogeneous ice structure. During the second step — primary drying — the frozen formulation is placed under a vacuum, initiating the sublimation of ice. During this process, ice crystals transition directly to vapor, which is then removed by a condenser. This is the most time consuming process. Finally, in the third step — secondary drying — the remaining water is removed by desorption when the temperature and pressure are increased and decreased, respectively.

The trick is to exclude unnecessary excipient and keeping it simple to achieve a stable cake structure, while also preventing agggreation and execesevely high viscosity. Two review papers from Kollár and Hsein et al. have compiled tables with common excipients and their associated functions (see tables in **References**)[3,14]. For example, sucrose and polysorbate 20 work as cryoprotectants and surfactant, respectively. Almost all excipients contribute to viscosity, but some amino acids e.g. histidine, proline, and arginine can attenuate this. Sucrose has another function, a structure giving feature important for the cake building (See section **Structured Literature Review**).

Quality evaluation

Quality evaluation of Trastuzumab involved monitoring critical parameters, including concentration, aggregation, and pH range that we controlled experimentally during the project, along with visual inspection and reconstitution time.

A consistent and acceptable reconstitution time is important for ensuring that the formulation is practically feasible for administration. Likewise, the concentration must be adequate for efficacious treatment. The Trastuzumab concentration can be calculated using Beer-Lambert equation adjusted to the molecular extinction

coefficent, ϵ, of Trastuzumab equal to 1.43547 mL/mg.cm when measuring the UV-Vis absorbance of a dilute solution.

At 280 nm, a wavelength at which proteins, including Trastuzumab, exhibit strong ultraviolet light absorption. The Beer-Lambert law was applied to calculate the concentration, where absorbance (A) is directly proportional to the product of the molar extinction coefficient (ϵ), path length (l), and concentration (c) through the equation $A=\epsilon \cdot l \cdot c$. This approach ensures precise quantification of Trastuzumab in solution.

Dynamic Light Scattering (DLS) can be used to assess the amount of aggregation, which in turn provide the the size distribution to identify any aggregate species in the sample solution by analyzing the scattering of light as particles move due to Brownian motion.DLS provides valuable insights into the homogeneity of the sample, with aggregates typically showing larger size distributions compared to monomeric species. controlling aggregation maintains the quality as it can affect the Trastuzumab stability and efficacy.

pH titration of Trastuzumab will help determine the range it will remain stable and unaffected by the conformational change during freeze drying and other mechanical and physicochemical stress. Proteins are inherently sensitive to pH change and by titrating a Trastuzumab sample with base or acid base, potential aggregation or conformational change can be recorded by UV-Vis and Fluorescence sensors.

The key factor, viscosity can be indirectly assessed using a texture analyzer. This instrument measures the force required to advance a syringe piston containing the formulation, generating a force-displacement curve. A linear relationship between force and displacement indicates Newtonian fluid behavior. This test is also known as glide force

5 Structured Literature Review

A search prompt with relevant keywords such as lyophil* and high-content* was structured with both primary articles and reviews in English and published after 1994. The final 13 papers subjected to reading were from 2019 and later. A python

application developed by Stephen Burleigh, bioinformatician at Lund Division of Food and Pharma was employed for the subsequent filtering after the Scopus search. Full Scopus query, and filter results are shown in **Appendix A** as well as VOSviewer and Scopus metadata

Key Findings

Through performing a Structures Literature Review (SLR), it was apparent some challenges of HC lyophilized protein formulations persist within the industry. Where cake stability[15,16], protein concentration[16], and viscosity (reducing agents)[17] were amongst the most frequently mentioned obstacles.

Cake stability is highly influenced by the protein concentration and choice of cryoprotectant/bulking agent (disaccharides) such as sucrose or trehalose. They stabilize the protein under stress, while also preventing aggregation and denaturing in their amorphous state. Crystalline mannitol, however, performs better in some cases, due to its semi-crystalline properties, greatly enhancing the sublimation rate under drying[16].

The sucrose and surfactant amount ought to be balanced towards the protein concentration, but are normally used in the range of 5 — 10% and ~0.01%, respectively. In several studies efforts were made to optimize these, which in turn affects pore size, surface viscosity, and crystallinity, all key factors in controlling reconstitution time[18–21]. In addition, Arginine was mentioned as a promising viscosity-reducing amino acid, capable of mitigating the high viscosity in injectable drugs.

Finally, future studies should investigate controlled nucleation and sublimation and excipients that behave well in reconstitution[22,23]. Scopus metadata about the literature review can be seen in Appendix A.

6 Results and Discussion

6.1 Market Analysis

Our product targets national and regional healthcare systems and drug distributors, with pancreatic cancer patients as a secondary audience.

By formulating monoclonal antibodies (mAbs) for subcutaneous administration, we offer a less invasive option that is also more resource-efficient. Price is the primary concern for distributors, while patients have several needs and requirements comprising minimal pain and maximal convenience. To accommodate this, consideration has been taken into account regarding the pH of the formulation, viscosity, and route of delivery for our products. pH has been chosen as close to neutral as possible without being dangerously close to the isoelectric point of the API.

Subcutaneous administration has the potential to improve patient access, reduce hospital resource usage, and enhance patient quality of life compared to surgery, particularly for patients with limited resectability or other advanced diseases. Our approach allows patients to manage treatment more easily and offers an alternative to complex, high-risk surgical interventions, beneficial for both healthcare providers and patients[1].

As there is currently a lack of subcutaneous mAb formulations on the market to treat pancreatic cancer, the deadliest cancer, this product is a breakthrough treatment that redefines comfort and accessibility in pancreatic cancer treatment.

In April 2024, AstraZeneca and Daiichi Sankyo's Enhertu demonstrated significant efficacy across various tumor types based on phase 2 trials. Its approval as the first tumor-agnostic HER2-targeted therapy marks a transformative milestone for patients with HER2-positive solid tumors. Previously, HER2-targeted treatments were restricted to specific cancers, such as breast, gastric, and lung cancers. Enhertu currently provides targeted therapy for patients with multiple HER2-positive solid tumors, such as pancreatic cancer. However, routine HER2 testing in tumor types other than breast, lung, and gastric cancer is not yet standard practice. The lack of routine testing and limited off-label efficacy studies of this therapy poses a challenge to its wider use. Enhertu's intravenous administration also places greater resource demands on healthcare systems and increases provider costs[24].

Olaparib, marketed as Lynparza by AstraZeneca and Merck, is another targeted therapy designed for cancers with BRCA1/2 mutations, including ovarian, breast, prostate, and pancreatic cancers. Specifically, for pancreatic cancer, Olaparib is prescribed for patients whose disease has not progressed after at least 16 weeks of platinum-based chemotherapy. Olaparib is a PARP inhibitor that blocks the poly (ADP-ribose) polymerase (PARP) protein in DNA repair. By inhibiting PARP, Olaparib prevents cancer cells from repairing their DNA, ultimately leading to cell death.

Olaparib is taken orally multiple times a day, which is more convenient than intravenous therapy. However, oral dosing presents compliance challenges as patients may miss doses or experience complications when swallowing the medication, as well as the challenges of daily dietary considerations. In contrast, subcutaneous administration can be done quickly at home. In addition, the three-week treatment cycle is more convenient so that compliance can be significantly improved. Despite these challenges, the mechanism of action of Olaparib specifically targets cancer cells with BRCA mutations or other homologous recombination repair (HRR) gene abnormalities. This precision makes it practical for these genetic-specific patients[25].

Trastuzumab is widely used in HER2-positive breast and gastric cancers, providing a more comprehensive range of applications for HER2-specific cancer types than Olaparib, which is limited to BRCA or HRR mutation cancers. Enhertu further expands the field of HER2-targeted therapy and brings hope to a broader range of patients with solid tumors. However, the subcutaneous route of administration of Trastuzumab has apparent advantages in terms of convenience of use and saving medical resources.

Given the increasing incidence of pancreatic cancer, our potential customer base is significant, encompassing healthcare providers and patients. Customers seek treatments prioritizing minimal pain, convenience, and improved quality of life. While competitors offer practical solutions, they have limitations such as intravenous administration and specific genetic targeting. According to some of the latest studies, pancreatic cancer cells overexpress HER-2, and Trastuzumab has been widely promoted in the treatment of breast cancer, with wealthy safety data for reference.

This means that applying Trastuzumab to pancreatic cancer seems to be a very effective strategy.

Selling products at different stages carry varying levels of profit and potential risk. Selling formulations prior to clinical trials can significantly reduce expenditures and mitigate the financial risks associated with potential drug failure. However, completing clinical trials and securing FDA and EMA approval for a new drug (IND) could yield substantially higher returns compared to selling the formulation to other companies. It is fundamentally a balance between risk and reward. Confident in the potential of our product, we aim to secure additional investment to bring it to completion and retain the rights for final market operations.

Our product effectively overcomes these barriers by offering a less invasive, subcutaneous treatment option that enhances patient adherence and accessibility. This position positions us to meet the urgent needs of healthcare providers and patients in the fight against pancreatic cancer. We have developed the technology and now intend to license it to pharmaceutical companies who will handle manufacturing, distribution, and patient access.

6.2 IP Strategy

Since Genentech's patent for Trastuzumab expired in 2014 in the EU and 2015 in the US[26], we can now design and develop a targeted dosage form for Trastuzumab to treat pancreatic cancer. Roche has developed and patented a subcutaneous formulation of Trastuzumab for breast cancer[27]. However, our formulation is distinct, with differences in excipients and their proportions, ensuring it does not fall within Roche’s patent scope.

IP plays a vital role in business development, protecting product interests, and is essential for obtaining financing. Investors value IP portfolios because they can demonstrate competitive advantages and sustainable market potential. IP can be used as collateral to establish technological innovation and attract investment or loans[28].

Applying for a patent to protect the product formula secures exclusive rights for 20 years[29]. Highlighting the uniqueness of the freeze-dried formulation patent serves to prevent competitors from copying or imitating the technology. Trademark registration

can ensure the uniqueness of our brand, product name, and logo in the market while helping consumers identify the brand and increase brand awareness[30]. For technologies that cannot be patented, such as production processes, we will protect this critical information through trade secrets to ensure that competitors cannot quickly obtain our technology or trade secrets. Leaking of technology can be prevented by signing an NDA (non-disclosure agreement) with employees, partners, or suppliers[31].

We mainly consider two implementation models to maximize the benefits of patent and technology licensing: one is patent licensing, that is, cooperating with other companies to license their own patents to others through technology licensing agreements to obtain license fees or profits. The other is transferring patents or technologies to other companies or institutions to obtain financial return or equity directly[32].

When considering IP registration for subcutaneous Trastuzumab, we focus on regions with high market potential. The US remains the largest biopharmaceutical market, followed by the EU, which values high-quality medicines. The Asia-Pacific is a very promising market with a large population. However, we have proposed a targeted strategy due to the high cost of maintaining global intellectual property rights. We will prioritize patent ownership in the US and EU to obtain the best return on investment. We will also adopt cooperation with multinational or local pharmaceutical companies to manage costs while securing other market access through revenue-sharing agreements.

Our marketing strategy combines differentiation and IP licensing. We will create a unique product identity through patent protection and trademarks, highlighting our innovations. In addition, by partnering with MNC (Multi National Company), we can expand our market presence by leveraging their sales and marketing channels. Our revenue will come from licensing agreements while our partners manage distribution.

6.3 Regulatory Analyse

The final product will be a multi-dose pre-filled syringe (PFS), categorized as a combinational product according to the U.S. Food and Drug Administration (FDA), as the auto-injector pen is intended for one specific drug, Trastuzumab[33,34]. This elicits

the need for a New Drug Application (NDA), the most comprehensive authorization in the FDA. Because this is a new way to deliver Trastuzumab, it is treated as a completely new drug by the FDA. This means it needs to go through an entire approval process, just like a brand-new drug would. The responsibility of compiling such an application belongs to the sub-ordinary licensing partners producing the final product. As formulators, we are responsible for providing the necessary information related to that formulation.

As the product is nearly a biosimilar to Herceptin® SC the marketed drug of Genentech Gmbh (part of Roche group) with Trastuzumab as API[35], the market authorization could be achieved with an Abbreviated New Drug Application (ANDA), and preclinical (animal experiment) research data can be omitted[36].This is already clear through the Herceptin® SC product. However, the indication of our product is pancreatic cancer, which is different from Herceptin® SC. Therefore, the required new drug investigational (IND) application still needs to be performed. The clinical trial data in pancreatic cancer patients need to be evaluated to confirm the efficacy of the formulation in pancreatic cancer patients.

A central difference in market approval and clinical trial authorization lies in the distinct nature of biosimilar drugs and generic small organic molecules (SOMs). Biosimilar drugs derived from living cell lines are inherently immunogenic. Their production is process-dependent, resulting in unstable and complex molecules that are difficult to characterize fully. This contrasts with SOMs, which are chemically synthesized, possess well-defined structures, and exhibit stability and complete characterizability. Consequently, biosimilars require a stricter safety margin, assessed by the Minimum Anticipated Biological Effect Level (MABEL), while SOMs utilize the No Observed Adverse Effect Level (NOAEL). These distinct safety standards are reflected in FDA and EMA testing guidelines[37,38].

Nevertheless, as described above, the product should still be pre-approved in the EU and the US, as the final product is a combination drug . The question is how extensive the documentation should be[26,36,39–43]. For a general device, the risk assessment associated with it determines its class and, thus, the pre-approval process. The FDA categorizes the process as follows. Premarket Notification (PMN) is for Class II devices, and Premarket Authorization (PMA) includes a Clinical Trial Exemption

(IDE) for Class III devices, where the higher the class, the higher the risk associated with the device.

Furthermore, licensing partners must, in addition to the above, adhere to the EU Regulations on Medical Devices (Regulation 745/2017)[44], as well as guidelines for biologics, ICH Q6[45]. Implementation of these ensures the safety and efficacy of the device and API, respectively.

Guidelines are recommendatory, but agencies like the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), and third-party auditors strongly advocate adherence. In contrast, regulations, such as Regulation 745/2017, are legally binding. Non-compliance with regulations constitutes a felony, resulting in market bans for the drug, whereas not following guidelines complicates third-party audits, requiring additional procedural documentation.

As outlined in our marketing strategy, our global export ambitions necessitate compliance with directives and regulations across all target markets—not just the Swedish market regulated by the Swedish Medical Products Agency (Läkemedelsverket), but also the European and North American markets under EMA and FDA oversight. ICH guidelines, harmonized with both FDA and EMA standards, are integrated into the master quality assurance plans of our organization, subcontractors, and licensing partners.

A quality management system is essential and encouraged by all regulatory agencies. By implementing the *Pharmaceutical Quality System ICH Q10*[46]*,* we show that quality control is commenced orderly, all activities are well documented and safely stored, and equipment validation routines and protocols for deviation exist. This ensures the traceability of the product chain, thereby ensuring the feasibility of managing deviations and product recalls.

To demonstrate that Trastuzumab is manufactured with the required safety and efficacy standards, we and our subcontractors adhere to Good Manufacturing Practices (GMP)[47]. Additionally, ICH guidelines provide international standards relevant to producing high-concentration lyophilized Trastuzumab, including process validation[48], stability testing[49], and risk management[50]. These protocols ensure

production quality, assess biological stability, and identify hazards using mitigation strategies.

6.4 Product design

To improve patient compliance, an auto-filled injector will be employed. The freeze-dried product will be securely stored in a sealed compartment, separate from the solvent in another compartment. Before injection, the solvent will be transferred into the compartment with the powder, allowing for complete and efficient reconstitution. Our original product are two vials, as shown in *Figure 1*. One is a lyophilized preparation of 60 mg Trastuzumab API, and the other is 2 ml of 0.9% Sodium Chloride Injection.

Figure 1 *Vials with high-concentration Trastuzumab and saline solution for injection. Picture generated by ChatGPT.*

Storage conditions:

Our product must be stored in a refrigerator at 2°C and 8°C [51].

Ingredients:

Each vial of product contains 60 mg of the active ingredient Trastuzumab and a 2 ml bottle of ml of 0.9% Sodium Chloride Injection.

It also contains;

- Histidine hydrochloride monohydrate buffer

- Proline
- Sucrose
- Polysorbate 20

The Trastuzumab protein is made using Chinese hamster ovary cells. Each excipient is carefully designed to ensure product stability, efficacy, and usability in Trastuzumab's high-concentration lyophilized subcutaneous formulation. Polysorbate 20 is used as a surfactant in the formulation to prevent protein aggregation and denaturation during lyophilization and reconstitution, thereby enhancing overall stability. Sucrose is used as a cryoprotectant and stabilizer to protect the antibody from stress during freezing and drying while also helping to form a stable bulk structure. Proline is used to reduce the viscosity of the reconstituted solution, which is critical for high-concentration formulations to facilitate subcutaneous administration. Histidine acts as a buffer to maintain the pH value in the optimal range and maintain the antibody's biological activity during its shelf life. Finally, 0.9% sodium chloride solution is the reconstitution solvent, ensuring isotonicity compatible with physiological conditions, minimizing irritation upon injection, and optimizing patient comfort.

Figure 2 *Molly 2.25 mL disposable autoinjector developed by SHL Medical, featuring ergonomic design and advanced functionalities for subcutaneous administration. (Source: SHL Medical, shl-medical.com)*

Our final product will be a PFS where our drug is pre-filled into a syringe and the patient reconstitutes the drug for direct use following the instructions. *Figure 2* shows SHL Medical's PFS as an example, which key features include a pre-attached needle,

two-step operation, audible/visual feedback, and a robust anti-roll design. Suitable for biologics requiring higher injection volumes. Unlike intravenous administration of Trastuzumab, the dosing of subcutaneous administration does not depend on body weight, and the treatment effect of a fixed dose of 600 mg is not inferior to that of intravenous administration and the frequency of administration is the same as that of intravenous injection, every three weeks[52].This means a fixed dose of PFS without designing different doses based on patient weight will suffice and won't increase the frequency of administration.

Using SHL Medical as a subcontractor to develop a PFS is beneficial, not only from an economic point of view, as it involves substantial investments in production facilities to make these pens. It also requires qualified personnel to validate the process and implement quality systems (see section **Regulation**). In addition, partnering with an experienced drug delivery company like SHL Medical allows one to leverage their expertise and streamline the development process, ensuring efficiency and patient-centric solutions.

6.5 Proof of concept

6.5.1 Materials and methods

pH titration of Trastuzuman

Prior to commencement of any formulation work, the pH stability of Trastuzumab was determined. To study the conformational stability and aggregation behavior of Trastuzumab across a specific pH range, The Probe Drum pH titration mode was employed. By measuring the Trastuzumab using the calibrated pH meter. The titration was carried out by gradually adding a base, NaOH to increase the pH. The program was initiated to perform the titration, automatically recording pH and absorbance spectra at the desired wavelength in real time. Data collected was analyzed to generate a titration curve, identifying key pH points that provide insights into the conformational stability of Trastuzumab and the onset of aggregation. as shown in **Appendix C**.

Trastuzumab preparation

The provided Trastuzumab had a concentration of ~8.4 mg/mL, suspended in a buffer system (25 mM sodium phosphate and sodium acetate) and 120 mM sodium chloride. To obtain a purer API before freeze drying without those buffers, buffer exchange and dialysis was performed using a 20.000 Da cellulose membrane (Thermo Scientific Slide-A-Lyzer® 20K Dialysis Cassettes 20,000 MWCO No.66030). The dialysis buffer was exchanged approximately every second hour for 8 hours and then let to be overnight before frozen into aliquots. The subsequent experimental work and formulation design was performed using this dialyzed Trastuzumab with histidine monohydrochloride monohydrate buffer. Further concentration was achieved using Amicon® Ultra (10 kDa) centrifugal filters, which were able concentrate the Trastuzumab two fold to approximately 20.83mg/mL, compared to 11.98mg/mL before, after buffer exchange/dialysis alone. The latter mentioned step was performed only for the second batch of formulations with higher concentration.

Trastuzumab concentration was determined photo-spectrometrically (280 nm) using a Thermo Fisher NanoDrop (ND1000) and with Trastuzumab's extinction coefficient equal to 1.43547 mL/mg.cm[24]. Concentration was measured before and after freeze drying to assess potential loss (aggregation/denaturing).

Formulations preparation

The sets of formulations were designed for comparing the effect of amount of viscosity reducing agent (proline and histidine), surfactant (polysorbate 20), disaccharide (sucrose) in Trastuzumab concentration. As the higher concentration of the mab requires a higher amount of stabilizer, the increase of these will result in increase of viscosity as well as reconstitution time. We set those composition designtargets for reduction of reconstitution time and viscosity. The composition design is shown in **Table 1**.

Table 1 Sample preparation for first and second freeze drying batch before and after concentrating Trastuzumab, respectively.

Batch/ Trastuzumab		1/ ~ 42mg in total (12mg/mL)						2/ ~ 60mg in total (20mg/mL)	
Excipient	Amount	1	2	3	4	5	6	1	2
Tween 20	0.25ml	✔		✔					
Proline	0.046g	✔			✔				
Histidine	0.062g	✔				✔		✔	✔
Sucrose	0.040g	✔		✔	✔	✔	✔	✔	✔

Sample Freeze drying

Samples were freeze dried under a program designed by Shuai Bai in the freeze drier (Epsilon 2-6D LSCplus) as shown in **Appendix B**. This program consisted of 4 processes: precooling, freezing, primary/main drying, and secondary drying. First samples and trays were precooled at -40°C without vacuum to ensure uniform temperature distribution among the samples before initiating freezing. During freezing, samples were freezed at -40°C for 10 minutes without atmospheric pressure for sample to equilibrate and then extend freezing to 5 hours at the same temperature and condition to ensure complete freezing of sample and the formation of ice crystal. The main drying was performed in 3 substeps. Initially, 0.1 mBa atmospheric pressure was applied for 30 minutes while maintaining temperature at -40°C to initiate sublimation. Temperature was kept low to avoid sample collapse. Second step, temperature was increased to -10°C for 30 minutes and 0.1 mBa to enhance the sublimation, lastly, time was adjusted to 8 hours while maintaining the same temperature and vacuum to remove any moisture content. Finally, the secondary drying involved further increasing the temperature to 20°C, initially for 1 hour and then for 12 hours, under the same vacuum conditions 0.1 mBa, to remove bound water and achieve the desired residual moisture content, to form a cake.

Analytical work

Visual inspection was performed to assess if the reconstituted formulation would fall within specification, including color, precipitate and turbidity which indicates physical change of Trastuzumab or denaturation as well as contamination (See **Appendix E**) Additionally, The reconstitution time of Trastuzumab was monitored

by recording the time taken for the sample to fully dissolve when rehydrated from its lyophilized form with NaCl.

Dynamic Light Scattering (DLS) measures the size distribution of nanoscale particles by analyzing fluctuations in light scattering caused by Brownian motion, and it was performed by DynaPro Plate Reader III from WYATT. Smaller particles cause faster fluctuations, while larger particles cause slower ones. By detecting these fluctuations, the particle radius is determined. First, sample is reconstituted using 2 ml of NaCl and triplicates of sample are prepared then pipette 2ml into the 96 plate wells, ensuring no bubbles as they can scatter light and affect accuracy which can interfere with DLS measurement. Data can be found in **Appendix F**.

NanoDrop was also used to measure the concentration of Trastuzumab after reconstitution of the lyophilized preparation, in order to compare it with the Trastuzumab content before lyophilization, and evaluate the loss of Trastuzumab after lyophilization. The specific process was the same as 5.2 and the results can be seen in the **Appendix H**.

Glide force test was performed to assess the viscosity of the reconstituted formulations. The samples were transferred to a syringe, before subjected to testing in the texture analyzer. Travel distance was set to 10mm per run, 3 runs per formulation. Reported results are at 2mm travel distance (see **Table 3**). Raw data is provided in **Appendix G**.

6.5.2 Experimental Result

The prepared formulations were freeze dried and reconstituted in saline water, and this is followed by property analysis. Stability and structural alterations of Trastuzumab were studied through pH titration using a Prob Drum, along with measurements of fluorescence, UV-VIS absorbance, PH determination, and light scattering. The reconstitution time, coloration and clarity was under visual inspection. DLS, NanoDrop were used to measure particle size distribution and concentration of the reconstituted solution separately. Additionally, texture analysis was conducted to

evaluate the glide force, which represents viscosity of the reconstituted solution. All these detailed results are discussed in separate reports in Appendices.

Information of all formulations (both batch 1 and batch 2) are summarised in Table 3. In conclusion, among all the formulations, formulation 4, which combines sucrose, proline and polysorbate 20, is considered to be the best choice. This contributes to its high concentration Trastuzumab formulation due to its moderate loss of antibody in freeze-drying operation, low density and no aggregation. Although the formulation in batch 2 remains relatively stable, its viscosity appears to be excessively high in the texture analysis result, which was a result of contamination in the vials. This makes the result not accurate.

There are several limitations of the study which can be considered important for future research and improvement. First of all, the amount of excipients were selected based on literature findings but were not optimized. Previous studies suggest that varying the amount of excipients can significantly impact several factors, including cake properties, reconstitution time, and formulation stability and so on. Moreover, only one vial for each formulation was made, which means the result can be affected by random error. Additionally, further analysis of the formulation is recommended, including tests such as analysis by size exclusion chromatography, osmolarity test, assessment of excipient crystallinity using X-Ray Powder Diffraction (XRPD), and moisture content analysis. These analyses can offer valuable insights for determining the optimal amounts of excipients.

Table 3 *Summary table for formulation data comprising final concentration, viscosity measured by glide force, particle size with dynamic light scattering as a measure of aggregation as well as reconstitution time, including pH. Reconstitution time was the time it took for the freeze-dried cake to dissolve in saline buffer.*

Batch	Formulation	Concentration mg/ml	Viscosity level	Particle diameter nm (%)	Reconstitution time (min)	pH
1	**1**	19.7761	medium	13.7 (100%)	1	7.76
	2	22.7256	high	13.1 (97.10%) 3611.6 (2.90%)	1	7.56
	3	20.8402	highest	13.5 (100%)	1	7.47
	4	21.0746	lowest	14.4 (100%)	1	7.56
	5	20.0878	medium	2.5 (0.60%) 15.3 (95%) 2363.4 (4.40%)	1	7.83
	6	20.255	medium	13.3 (100%)	1	7.56
2	**1**	31.5184	medium	14 (100%)	2	7.83

6.6 Future continuous production

The process of producing our formulation can be divided into different parts - upstream processing, downstream processing, formulation and filling. First, we have the upstream process. The process begins with continuous cell cultivation in a perfusion bioreactor. In this setup, the CHO cells are cultured to produce Trastuzumab. The inoculations of the production bioreactors were carried out with cells from a perfusion seed. On a pilot scale, the seed bioreactor used was a wave‑induced bioreactor, meanwhile the production bioreactor was a disposable stirred tank system. The viable cell density from the cells from shake flask cultures that were inoculated in the respective N‑1 bioreactors expanded to an increase of 30-40 times over a course of 6–7 days.

The cells from the N‑1 bioreactor were transferred to the production bioreactor, where pH was controlled with automatic addition Na2CO3 or CO2. In addition, in the bioreactor, CO2 stripping was performed to keep the levels below a certain point. The DO was controlled by addition of oxygen. A antifoam C emulsion was regularly added, as an prevention for foam formation in the bioreactor. The clarified harvest was collected in a stirred hold‑up vessel which in turn is connected to the following downstream process.

The downstream process was performed in four steps. Protein A capture step run with a 3‑column PCC process, a solvent/detergent virus inactivation, a CEX step in

bind/elute mode; and an AEX step in flow‑through mode. The two last steps are referred to as the polishing steps and can be used for regulation of the characteristics of the mab, if any specific loading quality variables such as loading profile are desired. This manufacturing was a 19 days-process, whereas 30 g MAB/day was produced continuously[53].

Next, our produced MAB shall be formulated with excipients to ensure its stability and efficiency for treatitive subcutaneous use. For pilot scale, this formulation can be performed in a Continuous Stirred-Tank Reactor (CSTR), which is ideal for continuous processes. Buffers, excipients, and the monoclonal antibody (MAB) solution are fed into the CSTR at controlled flow rates. The CSTR ensures consistent mixing and formulation under sterile and closed-system conditions. The reactor maintains steady-state operation, with constant agitation and real-time monitoring of parameters such as pressure, temperature and pH[54].

The MAB solution is placed in the dialysis chamber, and a buffer solution with the desired pH is continuously circulated on the outside of the dialysis membrane. The MAB-formulation shall now undergo filling into vials with an automated aseptic filling line to ensure accurate dosing and minimized product loss. The filled vials are placed into a continuous freeze-drying unit, in order to further concentrate the formulation by removing water by sublimation, and produce a stable MAB-formulation. After the freeze-drying process, the vials would be sealed within the freeze-drying unit (under either vacuum or inert gas) in order to maintain sterility and prevent any reabsorption of moisture[55].

7 Highlights

Marketing

- Marketing should primarily focus on national and regional health care system systems and distributors, while also catering to the needs of the patient.
- In relation to communication, the emphasis should be on where we have a competitive edge to comparable drugs e.g. less invasive treatment with

subcutaneous administration, offering better comfort and accessibility in pancreatic cancer treatment.

- In addition, the product offers a better quality of life and improved treatment adherence, while easing the workload for health care systems.

IP Strategy

- Securing exclusive right to the formulation prevents competitors from copying the recipe. A formulation also attracts investors concerned with guarantee for return of investment
- In connection to marketing, a trademark registration will ensure the uniqueness of the brand, product name, and logo, increasing brand awareness and recognition.
- Patent rights in the US and EU will be prioritized to obtain the best return on investment.

Regulation

- The production will adhere to strict safety standards for biosimilar drugs, assessed by the Minimum Anticipated Biological Effect Level (MABEL), to ensure patient safety.
- The product will be pre-approved in the EU and the US, complying with all relevant regulations and guidelines for medical devices and biologics.
- A robust quality management system will be implemented, adhering to Good Manufacturing Practices (GMP) and ICH guidelines to ensure production quality and traceability.
- The final product, a multi-dose pre-filled syringe (PFS), is a new way of delivering Trastuzumab and most likely requires additional clinical trials, eliciting the need for authorization between an abbreviated and a New Drug Application (NDA) for market approval.

Design

- The final product consists of two vials—one containing lyophilized Trastuzumab API and the other containing 0.9% Sodium Chloride Injection.
- The formulation includes carefully selected excipients: Histidine hydrochloride monohydrate buffer, Proline, Sucrose, and Polysorbate 20, each ensuring product stability, efficacy, and usability.
- The product will be stored in a refrigerator at 2°C and 8°C to maintain its stability and efficacy.
- The product will be administered using a pre-filled syringe (PFS) with a fixed dose of 600 mg, providing a convenient and user-friendly delivery method.
- The PFS will be developed in partnership with a subcontractor, leveraging their expertise and streamlining the development process for efficient and patient-centric solutions.

Proof of Concept

- The experimental work successfully demonstrated the stability and structural integrity of the lyophilized Trastuzumab formulation through various analytical techniques.
- The stability of API Trastuzumab within a certain pH range was verified through experiments and the feasibility of the preparation was confirmed.
- The formulation showed a high concentration of Trastuzumab, low density, and no aggregation, confirming its suitability for subcutaneous administration.
- The study identified formulation 4, which combines sucrose, proline, and polysorbate 20, as the best choice due to its excellent characteristics.
- The reproducibility of the freeze-drying and reconstitution operations of high-concentration Trastuzumab was verified through experiments.
- The proof of concept highlights the potential of this lyophilized HC mAb formulation as a viable and effective treatment option for pancreatic cancer.

Continuous Production

- The production process can be divided into upstream processing, downstream processing, formulation, and filling, with continuous cell cultivation in a perfusion bioreactor for efficient production.
- The downstream process involves four steps, including protein A capture, virus inactivation, and polishing steps, ensuring the quality and purity of the mAb.
- Formulation will be performed in a Continuous Stirred-Tank Reactor (CSTR) for consistent mixing and formulation under sterile conditions.
- The process includes automated aseptic filling and continuous freeze-drying to maintain sterility, prevent moisture reabsorption, and ensure product stability.

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10 Appendix

Appendix A Structured Literature Review

Table A1 *Scopus Search query and subsequent Python filtering results*

Query	URL for Scopus Search
TITLE-ABS-KEY ((lyophil* OR freeze*) AND ("high-concent*" OR "humanized monoclonal antibody") AND (formulation OR "drug product") AND ("Pre-formulation" OR formulation OR protein OR instab* OR characteri* OR degrad* OR surfactant OR stabilizers OR kinetics OR aggregat* OR viscos*)) AND PUBYEAR > 1994 AND DOCTYPE (re OR ar) AND LANGUAGE (english) AND NOT TITLE-ABS-KEY (("carrier" OR "biofilm" OR "impurities" OR "particles" OR "subvis" OR "nano"))	**https://t.ly/UqAcg**

Table A2 *Filtering terms used used in python application (Stephen Burleigh)*

Primary	Secondary	Excluded
protein	lyophiliz*	using
	aggregat*	
	viscos*	

Table A2 *Python application results with 98 articles and review at start and 13 at the end which were read (Stephen Burleigh)*

Filtering table

Filter	Remaining	Percent Left
At start	98	100.0
No duplicates	90	91.8
Has abstract	85	86.7
Has DOI	84	85.7
Has year	84	85.7
Has primary keywords	46	46.9
Has secondary keywords	34	34.7
No exclusion keywords	17	17.3
Adequate citations	16	16.3
On Topic	13	13.3

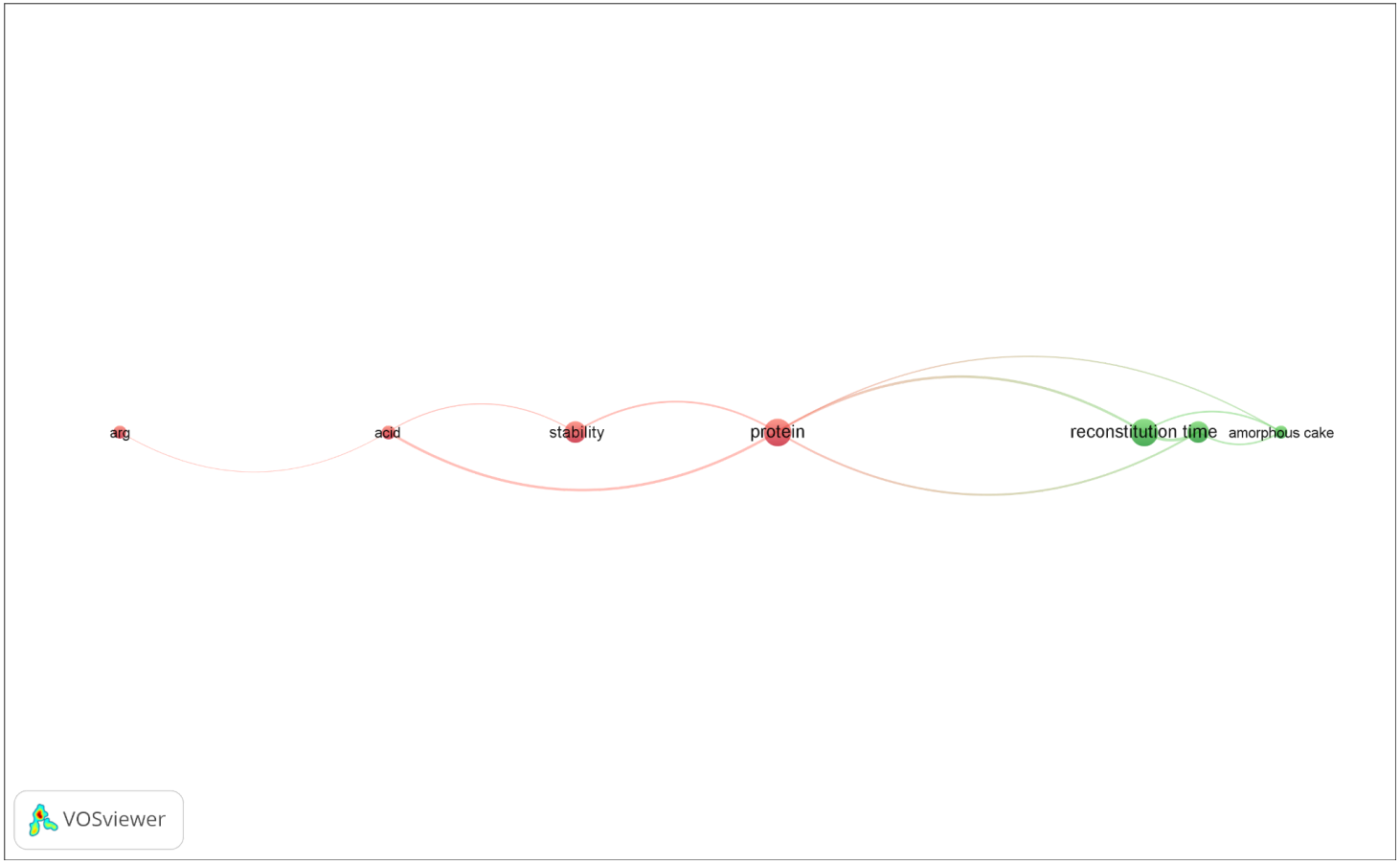
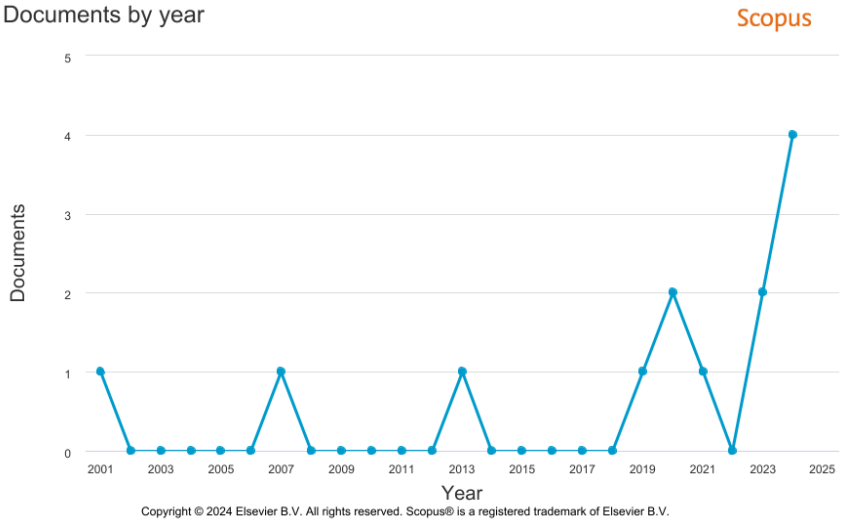


Figure A1 *VOSviewer Search Term Analyzer Function where the words used more than 7 times are shown*


Documents by year
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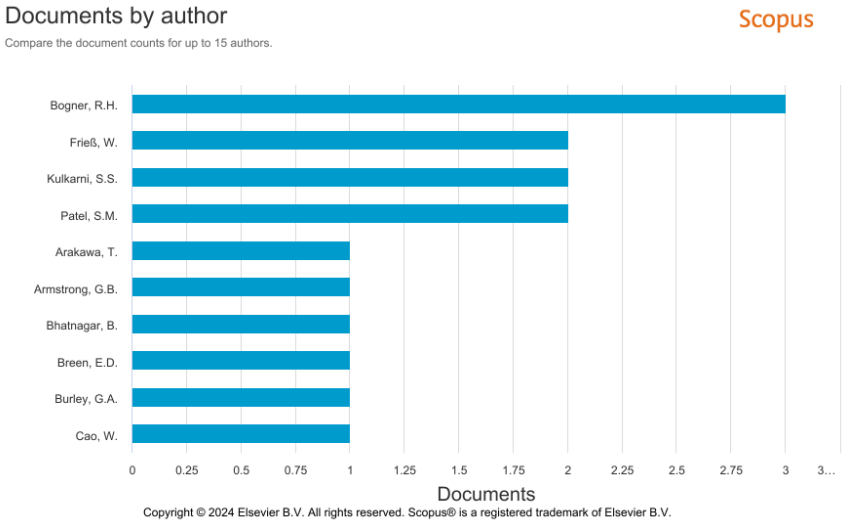
A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Frieß, W.
Kulkarni, S.S.
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Armstrong, G.B.
Bhatnagar, B.
Breen, E.D.
Burley, G.A.
Cao, W.
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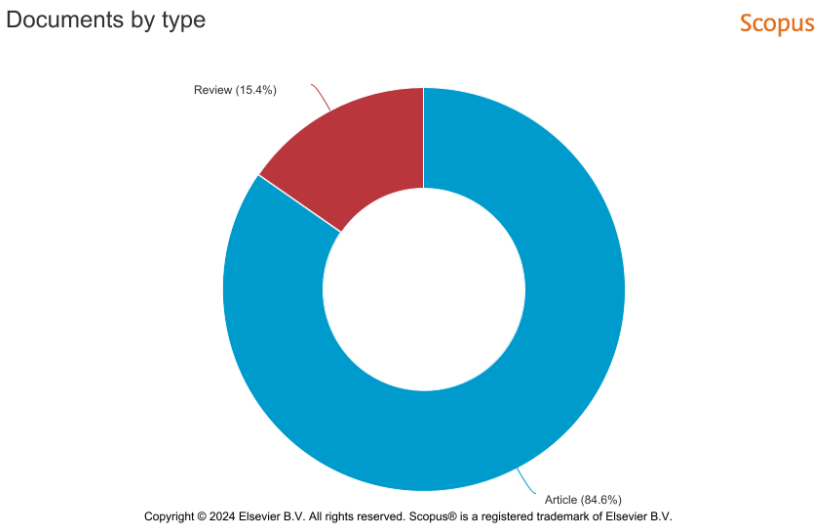
B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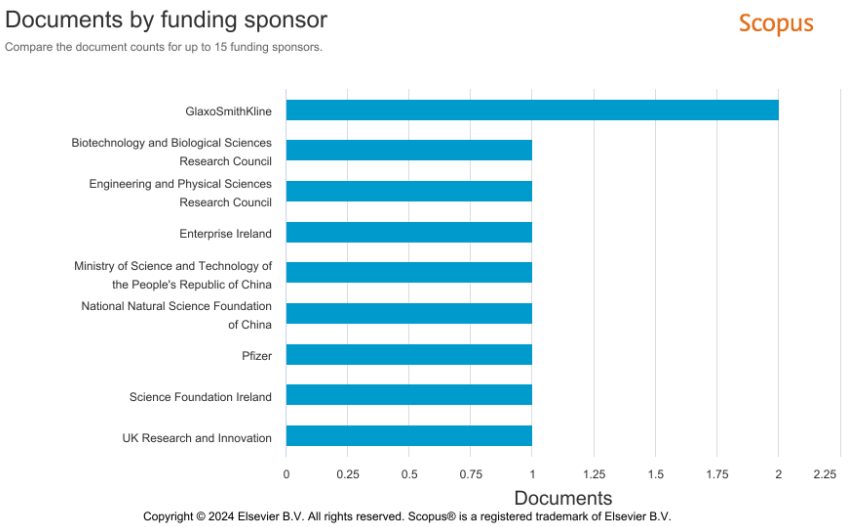
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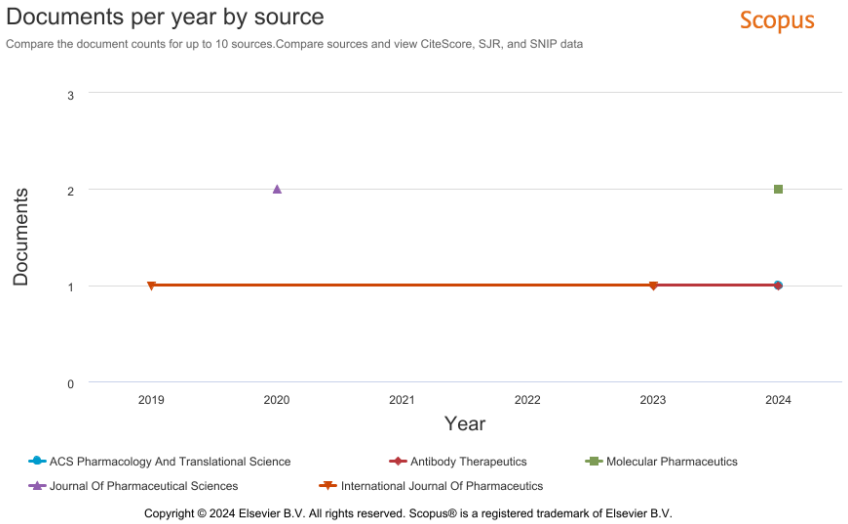

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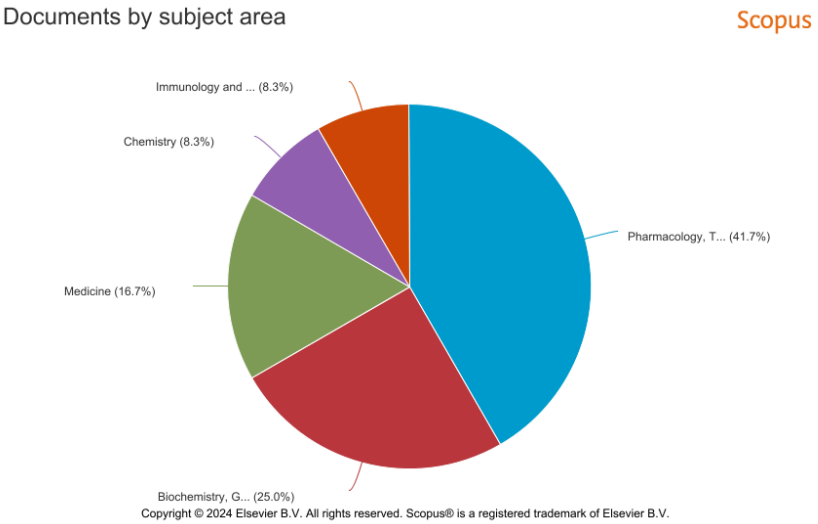


F

Figure A2 *Summarized literature search results from Scopus A - C sidpladisplaying findings for publication year, author, and type article. D - F shows the sponsors, journal by publication years and finally subject are in respective order.*

Appendix B Materials and Methods

Table B1 *Chemicals used in formulation preparation*

CAS	Name of compound
180288-69-1	Trastuzumab
7732-18-5	Purified water
7647-14-5	Sodium chloride (NaCl)
7647-01-0	Hydrochloric acid
1310-73-2	sodium hydroxide (NaOH)
147-85-3	Proline salt
71-00-1	Histidine
57-50-1	Sucrose
9005-64-5	Polysorbate 20

A

Section		1	2	3	4	5
Process phase		Precooling	Freezing	Freezing	Main drying	Main drying
Time	hh:mm		0:10	5:00	0:30	0:30
Temperature	°C	-40,0	-40,0	-40,0	-40,0	-10,0
Vacuum	mBar				0,100	0,100
Safety pressure	mBar				OFF	OFF
ΔT shelf	°C		OFF	OFF	OFF	OFF
ΔT product	°C		OFF	OFF	OFF	OFF
LyoControl-RX	%		OFF	OFF	OFF	OFF

B

Section		4	5	6	7	8
Process phase		Main drying	Main drying	Main drying	Secondary drying	Secondary drying
Time	hh:mm	0:30	0:30	8:00	1:00	12:00
Temperature	°C	-40,0	-10,0	-10,0	20,0	20,0
Vacuum	mBar	0,100	0,100	0,100	0,100	0,100
Safety pressure	mBar	OFF	OFF	OFF	OFF	OFF
ΔT shelf	°C	OFF	OFF	OFF	OFF	OFF
ΔT product	°C	OFF	OFF	OFF	OFF	OFF
LyoControl-RX	%	OFF	OFF	OFF	OFF	OFF

C

Figure B1 *Freeze-drying program used,* ***A)*** *Process Monitoring Chart,* ***B)*** *Precooling and Initial Freezing Phases,* ***C)*** *Main and Secondary Drying Phases.*

Appendix C Aggregation and conformational study

The Prob Drum pH titration mode was used to study the conformational stability of Trastuzumab within a certain pH range and whether aggregation would occur, in which the result illustrated that no aggregation appeared in the pH range of 2.5-10. Our final formulation has an average pH of 7.65, which is close to physiological pH (7.35-7.45), indicating potential stability and reduced risk of irritation upon administration, while also suggesting a suitable buffering capacity. The result aligns with the conclusion given by Patrick et al.[56], suggesting high concentration protein formulations have the potential to self-stabilize during freeze drying and storage without need for buffering agent (e.g., succinate, histidine, or citrate).

However, conformational change was observed with fluorescence within a wavelength range of 300-330 nm and pH of 8.52 (Figure C1). The possible explanation is the ionization of histidine (pKa 6.5) causes the alteration in the protein's electrostatic interactions. This conformational shift potentially disrupts the protein's stability and efficiency results in a reversible structural shift[57].

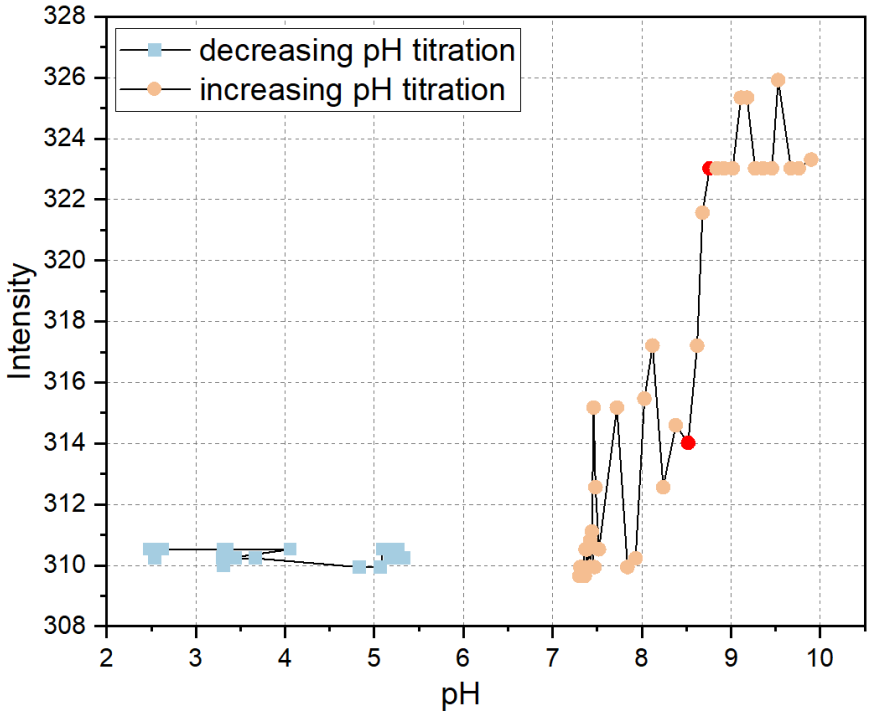


Figure C1 *Titration curve with fluorescence intensity within peak wavelength (300-330 nm) versus pH change, an obvious change in intensity was observed at pH 8.52, suggesting protein secondary structure changed.*

Appendix D Concentration

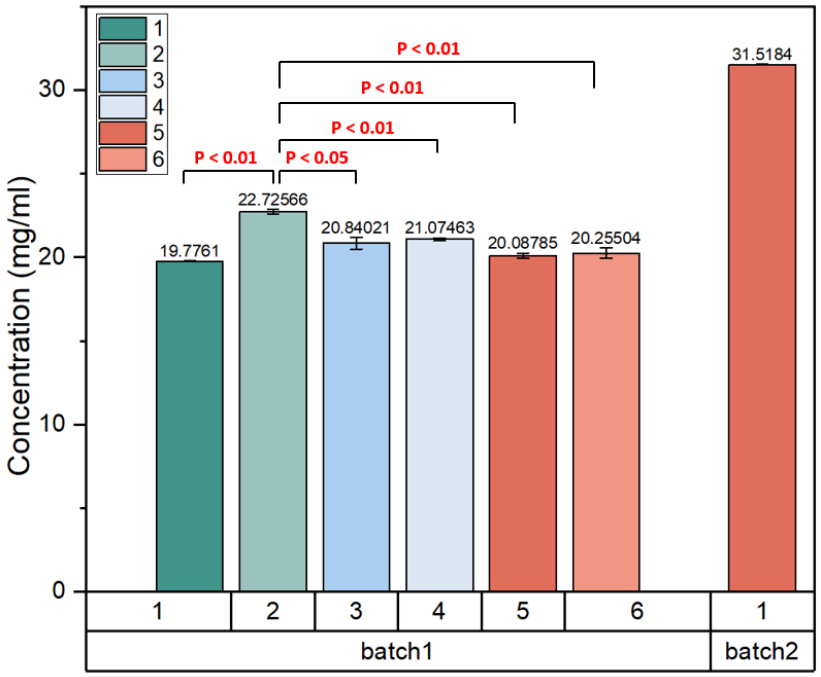


Figure D1 *Concentration of Trastuzumab in formulations after freeze drying*

Compared with the Trastuzumab concentration before freeze drying (11.98 mg/ml and 20.38 mg/ml respectively), the concentration of Trastuzumab increased approximately two-fold in both batches after the operation. Specifically, batch 1 reached an average concentration of 20.79 mg/ml, and batch 2 achieved 31.52 mg/ml. This increase suggests that the freeze-drying process is effective in concentrating Trastuzumab, which is essential for therapeutic applications requiring high-dose formulations.

In batch 1, compared to control (formulation 2), all other treatments cause significant reduction of Trastuzumab concentration. This illustrates that the addition of other excipients might cause loss of antibody during the freeze drying process. In batch 2, two technical replicates exhibit a similar concentration (not shown in graph), indicating an accurate measurement. A comparison of formulation 5 between batch 1 and batch 2 shows that the concentration increased by approximately 1.55-fold, indicating the concentrating operation is effective.

Appendix E Reconstitution, coloration, and clarity

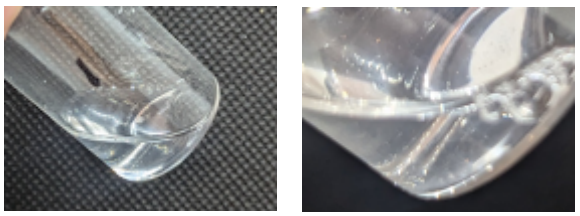
Reconstitution

The reconstitution time for lyophilized samples were recorded by watch and visual inspection. The average reconstitution time for batch 1 samples was 1 minute, whereas for batch 2 samples, it was 2 minutes. Some foam can be observed in the dissolving process of batch 2.

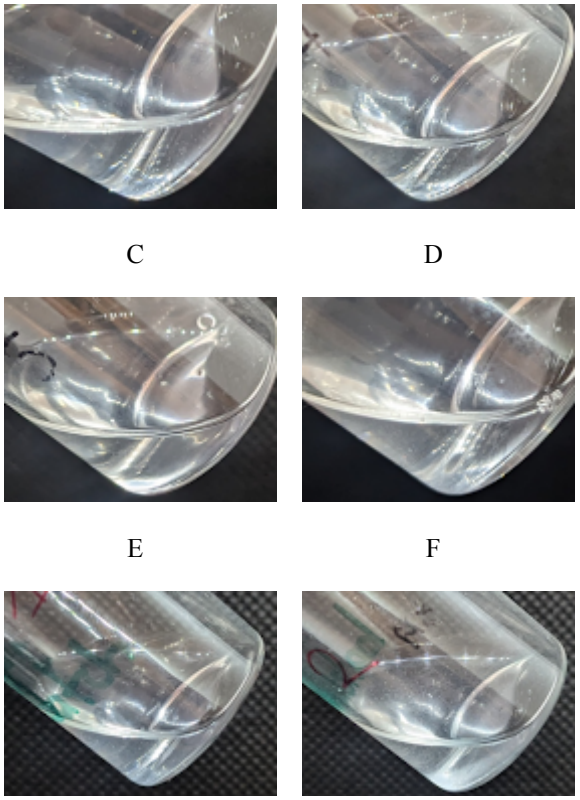
Although this study did not fully confirm the observation, it was noted that sucrose was difficult to dissolve in the histidine solution. A similar finding was reported and proven by Wenjin et al.[19]. , suggesting that while increasing stabilizer concentration is necessary for higher protein concentrations, it can also lead to increase of reconstitution times.

Coloration and Clarity

Clarity and coloration (method I and II) tests were performed on similar conditions described in the European Pharmacopeia 11.0 and the Apollo II light cabinet manual [58–60]. Within specification for reconstituted Trastuzumab was defined as "[...] free of visible particulates, clear to slightly opalescent and colorless to pale yellow"[61]. As seen in **Figure E1** and **Table E1**, None of the formulations were free from visible particles, although all could be assessed within the desired color. However, no comparison to color references was made, so the results are associated with considerable uncertainty as to whether these are within color specification. However, this was not investigated further.



A B



C D

E F

G H

Figure E1 *Images of reconstituted Trastuzumab showing (**A — F**) batch 1 formulation 1 — 6 and (**G — H**) batch 2 formulation 1 and 2.*

Table E1 *C larity (2.2.1) and coloration (2.2.1, method I-II) test according to European Pharmacopeia 11.0 with sample formulation and conformity status.*

Sample		Clarity	Coloration
Batch 1	1	DOES NOT CONFORM	CONFORMS
	2	DOES NOT CONFORM	CONFORMS
	3	DOES NOT CONFORM	CONFORMS
	4	DOES NOT CONFORM	CONFORMS
	5	DOES NOT CONFORM	CONFORMS
	6	DOES NOT CONFORM	CONFORMS
Batch 2	1	DOES NOT CONFORM	CONFORMS
	2	DOES NOT CONFORM	CONFORMS

Appendix F Dynamic Light Scattering (DLS)

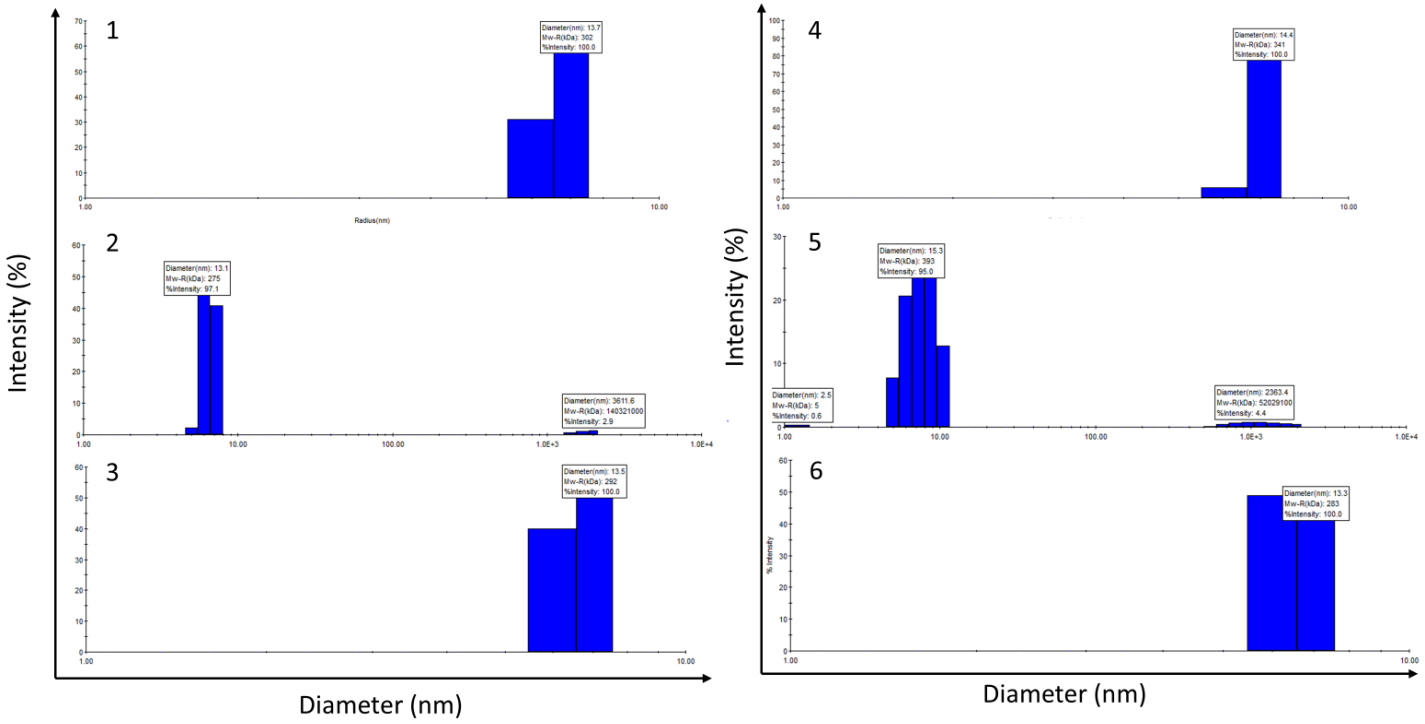


Figure F1. *Batch 1 size contribution from DLS*

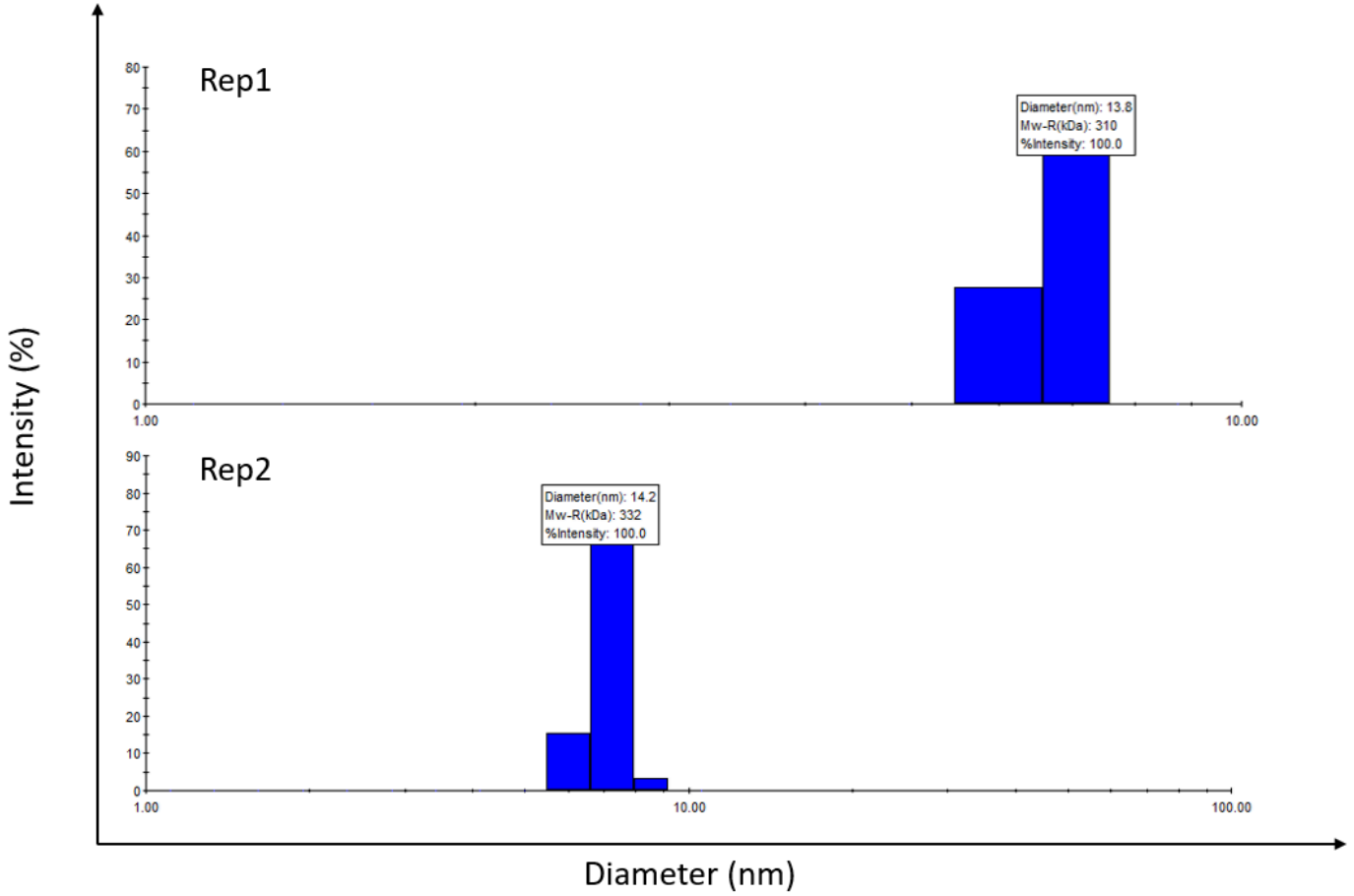


Figure F2. *Batch 2 size distribution from DLS*

The particle size measured by DLS (shown in Figure F1 and Figure F2 in Appendix F) shows an average diameter of 13.8 nm across all formulations in batches 1, except for formulations 2 and 5. These two formulations exhibit a small proportion of larger particles with diameter of 3611.6 nm and 2363.4 nm respectively, suggesting the presence of aggregation. Although in batch 2 both two replicates show no aggregation in average, large particles also appear in some of the replicates or acquisition for samples, indicating the reconstituted formulation solution is likely nonhomogeneous. Besides, it's noticed that the presence of large particles affect the model running in DLS, leading to less accurate results, but this is not further discussed in the report due to scope limit.

To conclude, the addition of histidine alone, or absence of excipients can lead to aggregation in the formulation during freeze drying. Additionally, the sample concentration appears to be closely associated with the occurrence of aggregation, this is also mentioned in other literature[4].

Appendix G Glide force/Texture analysis

Given that force at 2 mm gives the least variant results among measured forces, it was used to represent the viscosity of the samples (Figure G1). The box plot reveals that formulation 3 has the highest viscosity, while formulation 4 in batch 1 has lowest viscosity. The comparison among formulations 4, 5, and 6 indicates that the addition of proline and histidine effectively reduces the sample's viscosity, with proline demonstrating better performance. This aligns with the previous study by Monika et al.[62], suggesting proline can reduce viscosity through hydrophobic interaction with hydrophobic regions of mAbs and shielding the electrostatic interactions. Additionally, the comparison between formulation 1 and 3 illustrated that the combination of proline and histidine can also effectively reduce viscosity.

Samples from batch 2 have large variance in the raw data, suggesting the result might be affected largely by the foam formed during the measurement, resulting in data inaccuracy in this batch.

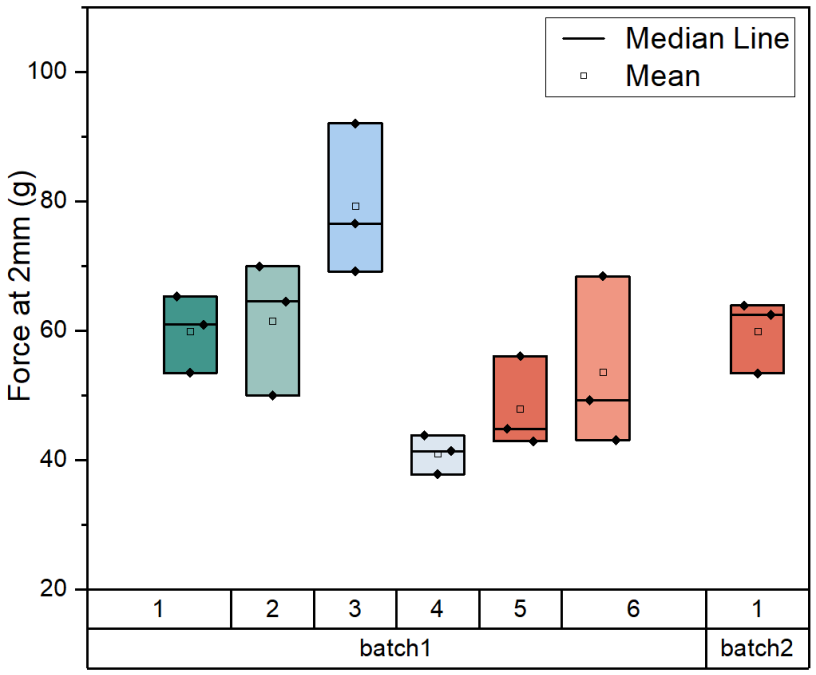


Figure G1 *Box plot for force at 2 mm in texture analysis, replicate contains large variance in batch 2 was discarded as outlier.*

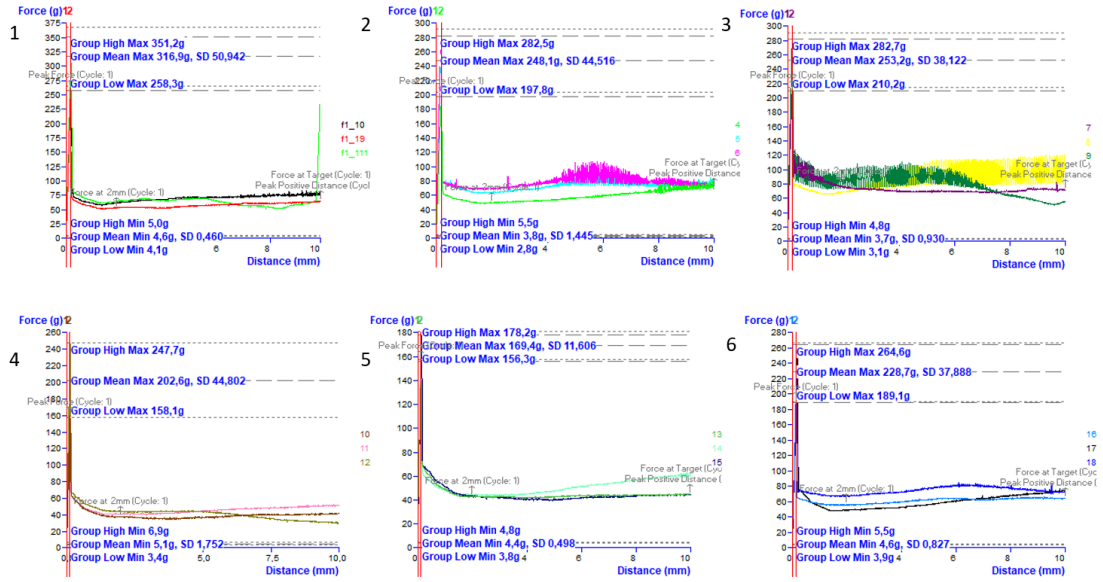


Figure G2 *Texture analysis raw data graph for batch 1 formulation*

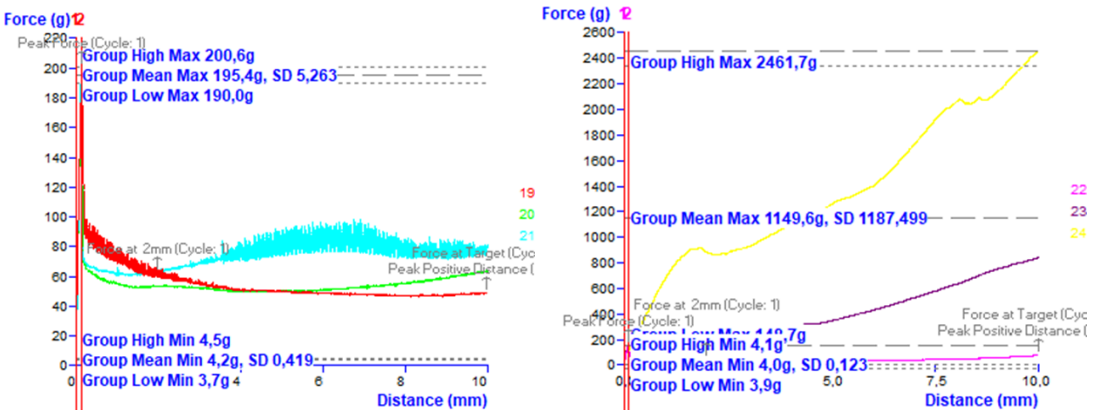


Figure G3 *Texture analysis raw graph for batch 2 formulation, the right one was discarded in data analysis due to unreasonable data*

Appendix H Concentration

Table H1*: Trastuzumab concentration calculation after concentrated*

Absorbance	ϵ	b= 1 cm	c (mg/ml)	average of c (mg/ml)
29.26	1.43547	1	20.38	20.38
29.13	1.43547	1	20.29	
29.38	1.43547	1	20.47	

Table H2 *Trastuzumab concentration after reconstitution*

sample	Absorbance	ϵ	b=1 cm	c (mg/ml)	Average C (mg/ml)
		First batch			
F1-1	28.412	1.43547	1	19.79282047	
F1-2	28.364	1.43547	1	19.75938194	
F2-1	32.767	1.43547	1	22.82667001	
F2-2	32.477	1.43547	1	22.62464559	
F3-1	29.554	1.43547	1	20.58837872	
F3-2	30.277	1.43547	1	21.09204651	20.79324774
F4-1	30.339	1.43547	1	21.13523794	
F4-2	30.165	1.43547	1	21.01402328	
F5-1	28.666	1.43547	1	19.969766	
F5-2	29.005	1.43547	1	20.20592559	
F6-1	29.393	1.43547	1	20.47622033	
F6-2	28.758	1.43547	1	20.03385651	
		Second batch			
F1-1	45.236	1.43547	1	31.51302361	
F1-2	45.184	1.43547	1	31.47679854	31.51842254
F1-3	45.082	1.43547	1	31.40574167	
F1-4	45.473	1.43547	1	31.67812633	